STRUCTURAL CHANGES IN RENAL CORTEX IN EXPERIMENTAL SKIN BURN INJURY AND UNDER THE CONDITION OF INFUSION SOLUTIONSUSE

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Abstract: Aim of research is the establishment of structural features of the course of the adaptive-compensatory and regenerative processes in the cortical substance of the rat kidney in late terms after experimental burn skin injury and the use of HAES-LX-5 % is relevant for theoretical and practical medicine.

Materials and methods.The article presents and analyzes the results of an experimental study on the structural changes of the cortical substance of the kidneys in rats in late terms after burn injury of the kidney with the condition of administration of intravenous infusion of 0.9 % NaCl solution and complex colloid-hyperosmolar solutions of lactoprotein with sorbitol and HAES-LX-5 %. Comparative analysis showed that functionally different cells of the nephrons under the infusion of detoxification solutions in the conditions of development of burn disease die by necrosis, apoptosis and anoikis. Also in epithelial cells of nephron tubules there weremitophagy and mitoptosis.

Result. Mitoptosis in epithelial cells of rat nephron tubules with experimental burn skin injury was carried out in two ways, which are related to I – destruction of the outer mitochondrial membrane; II – preservation of the outer mitochondrial membrane and attachment of autophagic (mitophagic) mechanisms to release the cell from degraded mitochondrial material. In the first case, the mitochondria first condensed, after which its matrix swelled and the fragmentation of the cross occured due to the destruction of the cross compounds. Finally, the outer mitochondrial membrane broke and the remnants of the crystals (in the form of vesicles) went into the cytoplasm. In the second case, the mitochondria condensed, vesicular fragmentation of the sac occured, but the outer mitochondrial membrane retained its integrity and the mitochondria were absorbed by the autophagosome. Next, autophagosomes merged with lysosomes and formed autophagolysosomes, which, subject to effective content processing, were transformed into vacuoles. The latter were emptied by exocytosis and ensured the cell was free from degraded material.

Conclusions. Only a lactoprotein with sorbitol has a membrane-plastic effect aimed at strengthening the mitochondrial membrane, in part of the mitochondria of epithelial cells of nephron tubules is ultrastructurally increased by the electron density and thickness of all components of the mitochondrial membrane. The maximal membrane effect of the action of lactoprotein with sorbitol on the mitochondria manifests itself fourteen days after the experimental burn skin injury and gradually (twenty-one and thirty days later) disappears, which coincides with the improvement of the overall clinical condition. There is every reason to believe that increased structuralization of mitochondria is a safeguard for the spread of mitoptosis and mitophagy, the excessive nature of which can lead to cell death.

Keywords: skin burn injury, infusion solutions, Lactoproteinum with sorbitol, HAES-LX-5 %, structural changes, renal cortex.

1. Introduction

With deep and significant skin burns, structural and functional disorders occur in almost all organs and systems, leading to the development of burn disease. Among the factors of these changes of cells, tissues, organs and systems of the burned organism are imbalance of water-salt homeostasis and endogenous intoxication. Therefore, for optimal treatment and prevention of the effects of thermal injuries, it is quite reasonable to use infusion solutions to reduce the level of toxins in the body and normalize water-salt metabolism [1].

Among colloidal solutions, the group of hydroxyethylated starches claims to be in the first place, but there is debate over the appropriateness of using some of them in certain clinical situations. That is why there is a need to develop new colloidal solutions of this group and to comparatively study the structural manifestations of their action on the internal organs of burns. Meanwhile, in the scientific literature there are no data on the structural changes of the cortical substance of the kidneys and the course of its regenerative processes in the late period after burn injury of the skin under the conditions of infusion of a new colloid-electrolyte-hyperosmolar drug HAES-LX-5 % [2].

Research to establish the structural features of damages and compensatory-adaptive changes in the renal cortex of rats in the later stages after experimental burn the skin under the conditions of the intravenous infusion of isotonic NaCl solution and integrated hyperosmolar solutions (Lactoproteinum with sorbitol and HAES-LX-5 % was conducted 105 white male rats weighing 155–160 g [3].

Aim of research is the establishment of structural features of the course of the adaptive-compensatory and regenerative processes in the cortical substance of the rat kidney in late terms after experimental burn skin injury and the use of HAES-LX-5 % is relevant for theoretical and practical medicine.

2. Materials and methods

The research is a component of the research work of the Department of Human Anatomy of the BogomoletsNational Medical University "Morphological changes of functionally different organs in the conditions of experimental burn injury" (state registration number 0115U000010), as well as performed within the framework of joint research work (planned in accordance with the agreement on scientific cooperation between Bogomolets National Medical University and Vinnitsa National Medical University named M. I. Pirogov) "Experimental substantiation of the effectiveness of complex infusion drugs on the model of burn disease in animals", which is a fragment of the planned of research work "To create new complex colloidal blood substitutes of polyfunctional action and solutions for resuspension of red blood cells (laboratory-experimental substantiation of their use in transfusiology)" (KPKV6561040, state registration number 0107U001132).

The experimental animals were divided into 7 groups (fifteen animals per group): I – intact animals; II, III, IV – rats without thermal injury who underwent single intravenous infusion of isotonic NaCl solution, HAES-LX-5 % and lactoproteinum with sorbitol and once daily for the first 7 days, respectively, at a dose of 10 ml/kg; V, VI, VII – animals with burns, which, along similar lines, and the dose in the same mode conducted separate investigational solutions [4].

Animal retention and handling were carried out in full compliance with the requirements of the "General Ethical Principles for Animal Experiments", approved by the First National Congress on Bioethics (Kyiv, 2001), with strict adherence to the recommendations of the "European Convention for the Protection of Vertebrate Animals and Experimental Use "other scientific purposes", the provisions of the methodological recommendations "Preclinical study of medicinal products".

Keeping rats in experiments, removing the animals from the rest of the experiments and related procedures carried out in accordance with existing bioetic requirements.

Skin burn injury modeled by sprinkle during ten seconds before the pre-shaved rat body side surfaces 4 hot copper plates (two on each side, each area – 13.86 cm^2). The plates were heated by immersing them for 6 minutes in water at a constant temperature of 100 ^oC. Total area of burn injuries amounted to 21–23 % of body surface experimental rats, which is sufficient for the formation of burns II-III, accompanied by shock moderate severity [5].

Material for morphological studies was processed according to conventional methods. For histological examination, tissue sections were stained with hematoxylin-eosin. Ultra-thin sections were prepared on an LKB ultramicrotome, and examined and photographed using a PEM-125K electron microscope. Semi-thin sections were stained with toluidine blue and methylene blue azure II.

Images from histological specimens stained with hematoxylin-eosin were taken to a computer monitor using a MICROmed SEO SCAN microscope and a Vision CCD Camera. Morphometric studies were carried out using VideoTest-5.0, CAARA Image Base and Microsoft Excel on a personal computer. Statistical processing of the obtained quantitative data was performed using the software “Excel” and “STATISTICA” 6.0 using parametric methods. For all indicators the values of arithmetic mean (M), error of arithmetic mean (m) and standard deviation (σ) were calculated. The significance of the difference of values between the independent quantitative values was determined at normal distribution by the Student’s t-test. In all cases, n=30. Differences at $p<0.05$ are considered valid [6, 7].

3. Result

With the use of light and electron microscopy methods, as well as morphometry with subsequent statistical processing of the obtained parameters, for the first time we established the peculiarities of damage and compensatory-adaptive changes in the cortical substance of the rat kidney in late terms after experimental skin burns of II–III degree, under the conditions of intravenous infusion of isotonic sodium chloride solution and complex hyperosmolar solutions (Lactoproteinum with sorbitol and HAES-LX-5 %) [4]. Under conditions of infusion of isotonic sodium chloride solution, mainly necrotic changes of the cell occur, which are accompanied by the development of interstitial edema, the appearance of hemorrhages and lymphocytic infiltrates. Under the conditions of infusion of Lactoprotein with sorbitol and HAES-LX-5 %, the spread of destructive changes in the cortical substance of burned rat rats was inhibited and substantially altered in both temporal and spatial dimensions [8]. It is proved that infusion of applied hyperosmolar solutions provides inhibition of cell necrosis, thus suppresses the inflammatory response and promotes the limited, local nature of necrotic and apoptotic changes. It was found that in rats without burn of the skin occurs mitoptosis of isolated mitochondria in epithelial cells of nephrons. This mitoptosis can be defined as basic (unstimulated) mechanism of intracellular quality control structures by removing old and defective mitochondria [9, 10]. For the first time described a massive mitoptosis in epitheliocytes of tubules of nephrons in burned rats, which are a form of response to factors of burn disease on cells; a mitoptosiscan be defined as induced. Manifestations of its morphological differences are:

1) the large number of structurally distorted condensed mitochondria;

2) accumulation of autophagosoma;

3) association with a transparent vacuoles containing a large number heteromorphous of autophagosoma containing varying degrees of structural degradation [11].

For the first time found that the stability of the size and distribution of mitochondria in the cytoplasm of epithelial cells in tubules of nephrons of burned rats is impaired. Mitochondria are subject mitoptosis; the formation of new mitochondria by kidney formation and separation; mitochondria fuse with one another, forming a separate and integrated into the network giant branched “mitochondrial complexes.” This process is dualistic meaning: on the one hand – this is a manifestation of compensatory-adaptive reactions thereby increasing the resistance of mitochondria (**Fig. 1**); on the other – this leads to distortion and extinction primary-side folded rim that has a significant impact on the state of reabsorption [12].

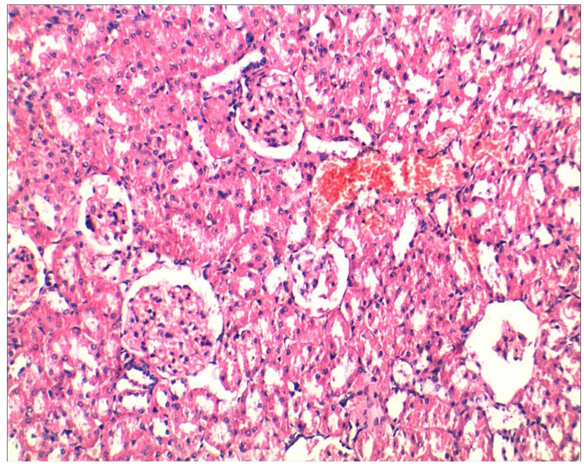

Fig. 1. Part in the cortex of the kidneys animals after 14 after in experimental skin burn injury of use HAES-LX-5 %. Microphotography. Reckoning gematoxillin-eozinum

Identified in two forms of mitoptosis in epitheliocytes nephron tubules of burned rats related to:

1) damage of external mitochondrial membrane;

2) preservation of external mitochondrial membraneand attracting autophagic (mitophagic) mechanisms.

In the first case mitochondria first condensed, followed by swelling of the matrix and fragmentation crista by the destruction of crista junctions. Finally, the external mitochondrial membrane ruptures and the remains of crista (as bubbles) out into the cytoplasm. In the second case,occurs mitochondria condensation and bubble fragmentation of crista, but external mitochondrial membrane rupture occurs, and mitochondria absorbed by autophagosome (or converted inautophagosome). Next autophagosome merge with lysosomes and education autophagolysosoma that under conditions of effective digestion content transformed into vacuoles. Last extrusion by exocytosis ensure the release of cells from the degraded material [13, 14].

4. Discussion and conclusions

The study has revealed one of the pathogenesis of complications of burn injuries of the skin, to determine the features of morphological changes of renal cortex and identify the positive impact of timely intravenous infusion combined with hyperosmolar solutions on the structure of one of the main organs for the removal of the toxins from the body, which is the kidney. It is proved that (unlike isotonic NaCl solution) Lactoproteinum with sorbitol and HAES-LX-5 % do specific cell protective effect on structure in renal cortex of burned rats, thus showing nephroprotective properties [15–17]. The Lactoproteinum with sorbitol specific action is to manifest for the first time the ultrastructural effect of enhancing mitochondrial epitheliocyte structuralization by increasing the thickness and electron density of all components of the mitochondrial membrane [18]. Strengthening of the mitochondrial membrane in some mitochondria is a regulator and protector of massive mitoptosis. The maximum effect is that most of the mitochondria within 14 days after the burn and gradually disappears, covering all of the smaller mitochondria, 21 days and 30 days after the burn (as the improvement in structural changes in the renal cortex and overall clinical condition of burned rats). Thus, it is a structural marker expression and "tense situation" in the cell, as well as a testimony to "improve this situation" (in this case, Lactoproteinum with sorbitol reveals his first-footed properties of the mitochohdrial tread).

Conflict of interests

No conflict of interest.

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